

Review

Genetically modified sunflower release: Opportunities and risks

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Abstract

Sunflower (*Helianthus annuus* L.) is a crop native to North America for which there are no genetically modified commercial varieties. Some of the transgenic traits incorporated in other crops have already been subjected to research and experimentation in sunflower. Several new traits have also been noted, with the most relevant of these being the aim to increase latex production. GM sunflower release would modify crop management through improved mineral nutrition, weed control, insect and disease resistance, and product quality. In this research, the traits investigated were reviewed and analyzed in connection with main crop constraints. These characters could potentially influence agro-ecosystem components and produce a significant environmental impact. In regions where sunflower coexists with wild relatives this situation could affect germplasm resources, with this being especially important at the centre of origin and where *Helianthus* populations established in Africa, Asia, and Europe. © 2006 Elsevier B.V. All rights reserved.

Keywords: Sunflower; Biotechnology; GM crops; Environmental impact; Herbicide tolerance; Pest control

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1. Introduction

Sunflower is the fifth most important source of edible oil after soybean, rapeseed, cotton, and peanut. The total world production of 25.8 million tonnes of sunflower seed go almost exclusively to oil extraction, providing 8.2% of total world

volume, estimated at around 107 million tonnes. The sunflower crop is important in several Eastern European countries and also in Argentina, which provides more than 10% of world production. Sunflower is considered good quality oil, but does not command the high prices of other edible oils, for which there is greater demand in the most select markets. The mean price of sunflower on the Rotterdam market over the last decade was US\$ 663 per tonne, exceeding those of soybean, palm, and coconut oils (FAS, 2005).

Biotechnology can speed up plant breeding, with many of the techniques complementing rather than substituting con-

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ventional methods (FAO, 2005a) and some biotechnological products have had a strong impact upon production systems because they have also facilitated crop management. This has led to a major increase in the total area devoted to genetically modified (GM) maize, soybean, cotton, and rapeseed production, which now exceeds 90 million ha, most of which are distributed amongst the 14 countries in which these crops have been authorized (James, 2005).

GM soybean constitutes a particularly significant case. Since its release as a commercial crop in Argentina 10 years ago, there have been increases in acreage, yield, and total production of 12.5%, 10.6%, and 25%, respectively (SAGPyA, 2002). This trend is still continuing and may, at least in part, be associated with RR (Roundup Ready[®]) soybean tolerance to glyphosate herbicide (Monsanto, 2002). This simplifies its cultivation under no-till systems immediately after wheat harvest, and greatly facilitates weed control. Moreover, it helps to reduce production costs, making the crop profitable in otherwise marginal areas of Paraguay, Brazil, and Bolivia. These facts help to explain the major increase in production observed in South America during the last 10 years (FAS, 2005). Sunflower and peanut are the only major vegetable oil yielding crops that have no GM varieties authorized for commercial use. This does not imply that versions of these products are not available through research; their use has been discouraged for other reasons. In the case of sunflower, the release of genetically modified organisms (GMO) must be carefully considered because of the agro-ecological implications of a possible transgene escape. This is an open-pollinated crop native to North America (Heiser et al., 1969; Harter et al., 2004) which has now wild relatives throughout the world's crop regions, Europe (Faure et al., 2002), Australia (Dry and Burdon, 1986), Africa (Quagliaro et al., 2001; Ribeiro et al., 2001), and Argentina (Poverene et al., 2002). Diffusion to these areas can be addressed to human activity. Crop-wild gene exchange allows transgene escape via gene flow in sunflowers (Whitton et al., 1997; Linder et al., 1998; Rieseberg et al., 1999; Burke et al., 2002). Transgenes from the crop could potentially disperse into wild or weedy populations enhancing their fitness and modifying their ecological interactions (Burke and Rieseberg, 2003; Snow et al., 2003). Conversely, wild or weedy sunflowers and volunteers can invade and interfere crop, and may modify traits, such as oil composition, via pollen flow (Faure et al., 2002; Bervillé et al., 2004). Furthermore, the impact of GMO release on edible oil marketing could be negative, because of the well known consumer resistance to GM products. So far, these circumstances have delayed the development of GM sunflower for commercial uses. The goal of this work was therefore to analyze the likely impact of the use of transgenic sunflower on agronomic crop management and to consider the possible consequences of authorization being granted for the commercialization of such products.

2. Registered GM crops

The level of adoption of GM crops in the USA is the highest in the world, with 49.8 million ha (James, 2005).

Their diffusion was preceded by intense research and development activity. At present, more than a hundred different GM products have been authorized for commercialization: 13 of these are crops, including maize, soybean, rapeseed, flax, and rice (Table 1). The products authorized for farming and industrial uses mainly facilitate weed, pests and/or virus control, and seek to improve quality and facilitate hybrid seed production. These commercial products represent the successful end products from just a few of more than 20,000 authorized trials undertaken with several dozen species.

The GM products available to farmers in Argentina have traits which facilitate crop management, such as herbicide tolerance and insect resistance. Only the soybean, maize, and cotton harbouring modifications of these traits have been authorized for commercial use (Table 1). In Argentina, the studied events include a smaller number of traits than in the USA. From the beginning of the biotechnological registrations in 1991, more than 800 cases have been authorized for research. Only about 10% of these cases involve sunflower, including modifications aimed at increasing the capacity for nitrogen assimilation and disease resistance. Even so, insect resistance and herbicide tolerance account for more than 80% of the release permits.

Australia, a country also actively involved in biotechnology, adds other traits to its GM products (Table 1). It has authorized the commercialization of transgenic maize, soybean, potato, and sugar beet, and an improved quality GM product, the high oleic soybean, is also now available. Authorized events for controlled research include metabolic transformation in photosynthesis, resistance to salinity, synthesis of new products (alkaloids) and modified quality in grapes, wheat, sugar cane, cotton, and flowers (carnation).

In spite of resistance to the use and consumption of GM products in Europe, a number of crops have already been authorized, and/or are currently under evaluation (Table 1). At present, commercial authorizations have been granted for the production and/or consumption of GM maize, rapeseed, endive, soybean, and flowers. The genetic modifications confer upon them similar traits to those previously detailed for the USA, Australia, and Argentina. Among products pending approval there is a variety of potato with modified starch content for industrial use. Amongst European states, Spain stands out as a major producer of transgenic crops, with over 100,000 ha of transgenic maize (James, 2005).

Although transgenic sunflower varieties have already been obtained, they remain the subject of ongoing research in both the USA and Argentina. Fig. 1 shows that the interest in GM sunflower research has decreased in the 21st Century, probably because official control bureaus have imposed restrictions in the face of ecological concerns. Although it is impossible to accurately assess the present extent of private research, public registrations on this crop include the traits detailed in Table 2. The main impacts upon crop management can be analyzed as follows.

Table 1
Traits on released GM plants for cultivation and/or consumption in four selected areas of the world with intense use of GMO

Intended effect (involved enzyme, gene, or agent)	Cultivation and consumption (food and feed)			Consumption EU ^d
	USA ^a	Argentina ^b	Australia ^c	
Tolerance to glyphosate (EPSPS, EPSPS + GOX)	Alfalfa (only feed), corn, cotton, creeping bentgrass (only feed), rapeseed, soybean, sugar beet, wheat	Corn, cotton, soybean	Corn, rapeseed, soybean, sugar beet	Corn ^c , cotton, rapeseed, soybean
Tolerance to glufosinate ammonium (PAT)	Corn, cotton, radish, rapeseed, rice, sugar beet	Corn	Corn, rapeseed, soybean	Corn, rapeseed
Tolerance to bromoxynil (Nitrilase)	Cotton, rapeseed		Cotton, rapeseed	
Tolerance to sulfonyleurea	Cotton, flax			
Resistance to Lepidoptera (Cry1F, Cry1Ac, VIP3A, Cry2ab, Cry1ac, Cry9C, Cry1Ab)	Corn, cotton, tomato	Corn, cotton	Corn, cotton	Corn, cotton
Resistance to Coleoptera (Cry3Bb1, Cry34Ab1, Cry35Ab1, CryIIIa)	Corn, potato		Corn, potato	Corn
Virus resistance (mop-top, Y, X, several mosaic)	Papaya, potato, squash		Potato	
Male sterility (Barnase); fertility restorer (Barstar)	Radish, rapeseed		Rapeseed	Rapeseed
Male sterility (DAM)	Corn			
Phytase degradation	Rapeseed (only feed)			
Increase lysine level (cDHDPS)	Corn			
High oleic oil content (GmFad2-1)	Soybean		Soybean	
High laurate oil content	Rapeseed			
Delayed ripening, two traits	Tomato			
Delayed ripening	Melon, tomato			

^a <http://www.cfsan.fda.gov/~lrd/biocon.html> (access June 2, 2006).

^b http://www.sagpya.gov.ar/biotecnologia/conabia/eventos_comerciales (access June 3, 2006).

^c <http://www.ogtr.gov.au/rtf/gmorec/gmfoodprod2.rtf> (access June 3, 2006).

^d http://ec.europa.eu/food/food/biotechnology/authorisation/index_en.htm (access June 3, 2006); http://ec.europa.eu/food/dyna/gm_register/index_en.cfm (access June 3, 2006).

^e By far, Bt Corn is the most widely GMO planted in EUA at Spain, France, Portugal and Germany (James, 2005).

3. Managing transgenic sunflower crops

3.1. Mineral nutrition

Sunflower is a highly nitrogen-dependant crop which, unlike soybean, does not perform nitrogen fixation. This limits its growth and development in poor soils and under no-till situations, where it is necessary to add nitrogen fertilizers (Díaz Zorita et al., 2003). Biotechnology for GM sunflower has been put forward as a possible way to improve nitrogen absorption.

In plants, ammonium absorption, which is an alternative pathway to the nitrogen cycle, is performed through the glutamine synthetase (GS) enzyme. However, in darkness and

with a low available C:N ratio, some variants of asparagine synthetase (AS) enzyme, coded by HAS1 and HAS1.1 genes provisionally store N as asparagine, thereby preventing ammonium intoxication (Herrera-Rodríguez et al., 2004). In GM plants, AS can substitute GS under conditions that limit its activity (such as in *Medicago truncatula*, Carvalho et al., 2000) and act as an alternative N-storing metabolic pathway (as in *Nicotiana tabacum*, Ferrario Méry et al., 2002). AS expression in GM sunflower might therefore improve N metabolism and contribute to a more efficient use of this element.

3.2. Production system

Sunflower has similar crop requirements to maize and soybean. It cannot be defined as highly tolerant to drought, but its ability to explore the soil profile helps it to survive under drought conditions better than many other species, if there is water available deep in the soil profile. It can be cultivated under conventional tilling, with reduced tilling or under no-till systems, but systems that compact soil should be avoided, because they limit plant growth (Blamey et al., 1997). The use of no-till in rotations including sunflower is highly recommended as it helps to maintain the soil structure due to the rapid decomposition of crop residue once it has been buried (Bowman et al., 2000).

In Argentina, no-till has been adopted by farmers on a large scale; in over 50% of the area devoted to grain production.

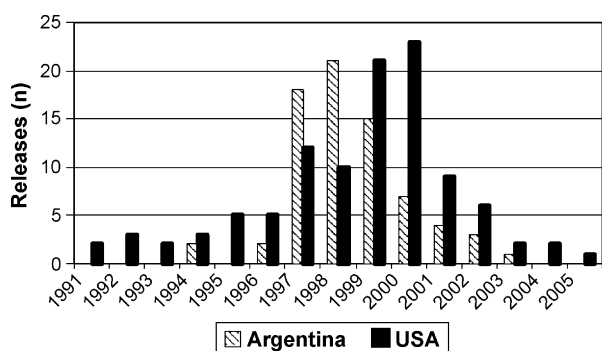


Fig. 1. GM sunflower release permits in the United States of America and Argentina since 1991.

Table 2
Expressed traits under field experimentation in sunflower

Intended effect	Responsible (Country)	Brief description
Tolerance to glyphosate	Monsanto (AR, US), INTA (AR)	5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) synthesis by expression of <i>Agrobacterium tumefaciens</i> genes
Tolerance to glufosinate ammonium	Zeneca (AR), Van der Have (NL)	Phosphinothricin acetyltransferase (PAT) synthesis by expression of <i>Streptomyces hygrosopicus</i> or <i>Streptomyces viridochromogenes</i> genes
Increased nitrogen assimilation	Zeneca (AR), Van der Have (US, NL)	Ammonium incorporation by Asparagine synthetase (AS) or increased N assimilation by nitrate reductase or nitrite reductase synthesis
Resistance to Lepidoptera	Mycogen (AR), Pioneer (AR), Van der Have (AR, NL), INTA (AR), Dow (AR)	Bt-derived insect resistance mediated by synthesis of endotoxins (Cry 1F) from <i>Bacillus thuringiensis</i>
Resistance to Coleoptera	Van der Have (US, NL), Zeneca (AG)	Cowpea (<i>Vigna unguiculata</i>) trypsin inhibitor synthesis (CpT1) plus Snowdrop lectin (NptII (SM))
Fungal resistance	Pioneer (FR, AR, US), Syngenta (US), INTA (AR) Zeneca (AR), Advanta (AR), Van der Have (NL)	Oxalate oxidase (OXO) synthesis by expression of wheat or barley genes conferring resistance to <i>Sclerotinia sclerotiorum</i>
Rubber yield increased	Colorado State University (US)	Enhanced quantity and quality of rubber production by expression of the synthesis complex of <i>Parthenium argentatum</i> (Guayule)
Enhanced protein quality	Pioneer (US), Van der Have (US)	Storage protein from <i>Bertholletia excelsa</i> (Brazil nut) with high methionine content.
Modified stearate content	Rustica Prograin Genetique (FR)	High stearate content. Reduction of stearic acid content
Others	Van der Have (NL, FR, SP, AR)	Albumin, asparagine, chalcone, chitinase, fructosyltransferase, glucanase or levan sucrose synthesis. Chlorsulphuron tolerance, fungal resistance, male sterility/fertility restoration, drought tolerance, marker system, MAC promoter
Broomrape control	Pioneer (SP)	No available information

Sources: <http://www.biotech.jrc.it/doc/snifs.rtf>, <http://biotech.jrc.it/deliberate/dbplants.asp>; <http://www.aphis.usda.gov/brs/status/notday.html>; <http://www.sagpya.gov.ar/biotecnologia/conabia> (access June 1, 2006). Country: AR = Argentina, US = United States, FR = France, NL = The Netherlands, SP = Spain.

Soybean is by far the main crop subject to this soil conservation system, being followed by maize and wheat. In contrast, sunflower accounts for less than 3% of the no-till area (AAPRESID, 2006). Difficulties associated with the use of postemergence herbicides to control weeds affecting sunflower could explain why no-till has not been adopted by many farmers cultivating this crop.

Compared to the glyphosate tolerant (RR) soybean, weed control under no-till for sunflower is more complex and not always very effective. Weed control under no-till could be improved by allowing sufficient time for preplant herbicide to take effect and by applying granular formulations (NSA, 2006). However, granular herbicides are expensive and farmers tend to resist their early application, usually preferring postemergence products. Many herbicides from that group are effective in controlling grass weeds but controlling latifoliate can only be achieved to a certain extent and through the application of a limited range of herbicides (ASAGIR, 2006; MAPA, 2006). These include acetonifene, which can only be used in early crop stages and which persists in the upper layers of the soil profile (Vischetti et al., 2002).

Although still not widely disseminated, the GM technology that has been developed for sunflower includes tolerance to glyphosate and glufosinate-ammonium herbicides. Both of these herbicides are systemic and neither has residual effects upon the soil. Glyphosate is used on a very large scale and is relatively inexpensive, but reiterated use can promote weed resistance (<http://www.weedscience.org/summary/MOASummary.asp>).

The need for RR sunflower to facilitate crop management in no-till systems seemed to disappear with the discovery of genes capable of conferring resistance to herbicides that belong to the imidazolinone (IMI) and sulfonyleurea groups and which were found – in wild sunflower populations in Kansas, under field conditions – to inhibit the hydroxyacetic acid synthetase (AHAS) enzyme (Baumgartner et al., 1999; Kolkman et al., 2004). By transferring these mutations to crop germplasm in the USA and Argentina, seed companies created non-GM sunflowers, under the commercial name of Clearfield, that were tolerant to both imazapyr and imazamox (Zollinger, 2003). Tolerance gene expression in these new varieties allows herbicide application at advanced stages of crop development, thus controlling the majority of weeds.

The hemiparasitic weed broomrape (*Orobancha* spp.) which constitutes an important crop limitation in the Mediterranean region could be effectively controlled in sunflower if herbicide resistant varieties were available. This strategy has proven useful in others crops (Nandula, 1998) and could be improved if the herbicide were brought with the seed, because broomrape affects the roots before emergence. At present, control strategies tend to use a specific gene mechanism which is also obtained in wild species (Fernández-Martínez et al., 2000; Labrousse et al., 2004). However, the continuous appearance of new races of the weed means that a process of constant renewal of resistance sources is required to maintain these control strategies. Some herbicides

that are members of the imidazolinone and sulfonylurea families including imazethapyr (Gressel et al., 1996) inhibit AHAS (Group B) and are therefore useful for controlling *O. aegyptiaca* and *O. cernua* (Nandula, 1998; Alonso et al., 1998). Some other groups have also proven effective against this weed, including glufosinate-ammonium (Valkov et al., 1998) and glyphosate (Collin, 1999). This may also be possible with GM sunflower because tolerance to these herbicides is currently under investigation.

There are many cases in the world of weed populations displaying resistance to herbicides that inhibit AHAS (95 cases in 63 genera, including *Helianthus*) and also to other herbicides; this points to the need to keep on searching for new control strategies. Table 3 shows selected cases of weed resistance to the chemical group of herbicides which could be used in sunflower, under different management strategies, including two GM varieties at present under research. Given the absence of glufosinate-ammonium resistance among weeds, a good long-term strategy could involve incorporating this tolerance through GM sunflower. Moreover, two homologous “bar” and “pat” genes that codify the phosphinothricin acetyltransferase (PAT) enzyme have been shown to be safe for this purpose as they do not cause allergy and are rapidly degraded in the intestine (Hérouet et al., 2005).

However, research and development should focus on more than simply obtaining broad spectrum herbicide-resistant sunflower. Science and technology policies should also outline and evaluate other integrated management strategies, which are rarely pursued by commercial companies which do not regard them as “retrieving technologies”. Without a doubt there is no single safe way in which to avoid potential problems associated with herbicide-resistant weed development: in agriculture, weed control should be a long-term strategy and involve the application of a number of different management techniques (Matthews, 1994).

3.3. Insect control

Crop insects present a different type of problem. At the centre of origin of sunflower, in North America, there are almost 50 species belonging to genus *Helianthus*. Almost 40% of at least 25 different insect species that constitute plagues for this crop are restricted to this genus. On the other hand, in Europe and South America most of the insects that affect sunflower are unspecific (Charlet et al., 1997). Of 16 pests reported during the last 5 years, three are restricted to the genus *Helianthus*, being found only in the centre of origin. The others are polyphagous and have a number of unspecific controllers, with the main cosmopolitan one being *Helicoverpa armigera* (Table 4).

One of the most generalized sunflower constraints caused by Arthropoda is stand establishment failure due to soil larvae: mainly of Coleoptera, Elateridae, and Lepidoptera. These herbivores which feed on seedling stems and roots at different levels all correspond to polyphagous species. Insects that eat the aerial parts of plants, including some aphids and white flies, can be particularly important during early stages of crop development. A small number of these predators are exclusive to sunflower and are only found at the centre of origin (Charlet et al., 1997; Lopez Bellido, 2002).

The relative importance of crop plagues constitutes a dynamic situation that technological developments can do much to change. This does not only relate to improved control methods but also to general changes in the ecosystem. With the increase in no-till surfaces, two previously unnoticed snails of genus *Deroceras* have recently become limiting factors for sunflower crops (Carmona, 2001).

Classical sunflower breeding techniques have succeeded in achieving resistance to the European moth (*Homoeosoma nebulella*) which was once the main constraint on the diffusion of this crop in Europe. The source of resistance was found in wild sunflower populations in North America. On the contrary,

Table 3
Sunflower postemergence weed control strategies and documented cases of resistance to the herbicide chemical group

Technology (availability)	Herbicides	Chemical group	HRAC group	Mode of Action	Resistant weeds: total number of cases and selected representative genera
Conventional sunflower (in use)	Aclonifen	Diphenylether	F3	Bleaching: inhibition of carotenoid biosynthesis	4: <i>Agrostis</i> , <i>Lolium</i> , <i>Poa</i> , <i>Polygonum</i>
Sunflower IMI Clearfield [®] (recently released in the U.S. and Argentina)	Imazapyr, Imazethapyr, Imazamox	Imidazolinone	B	Inhibition of acetolactate synthase or acetohydroxyacid synthase (AHAS)	95: <i>Amaranthus</i> , <i>Ambrosia</i> , <i>Anthemis</i> , <i>Avena</i> , <i>Bidens</i> , <i>Brassica</i> , <i>Bromas</i> , <i>Chenopodium</i> , <i>Conyza</i> , <i>Cuscuta</i> , <i>Cyperus</i> , <i>Digitaria</i> , <i>Diploaxis</i> , <i>Echinochloa</i> , <i>Eleusine</i> , <i>Euphorbia</i> , <i>Kochia</i> , <i>Lactuca</i> , <i>Lolium</i> , <i>Papaver</i> , <i>Parthenium</i> , <i>Phalaris</i> , <i>Raphanus</i> , <i>Sagittaria</i> , <i>Salsola</i> , <i>Setaria</i> , <i>Sinapsis</i> , <i>Sysimbrium</i> , <i>Sonchus</i> , <i>Sorghum</i> , <i>Stellaria</i> , <i>Xanthium</i>
Sunflower RR [®] (under research)	Glyphosate	Glycine	G	Inhibition of EPSP synthase	8: <i>Amaranthus</i> , <i>Conyza</i> , <i>Ambrosia</i> , <i>Eleusin</i> , <i>Lolium</i> , <i>Plantago</i>
Sunflower LL [®] (under research)	Glufosinate-ammonium	Phosphinic acid	H	Inhibition of glutamine synthetase	Unknown

Source: <http://www.weedscience.org/summary/MOASummary.asp> (access May 31, 2006).

Table 4
Host range, geographic area, controllers of sunflower pests, and number of references reported for the last 5 years

Common name	Species	Taxa	Host range	Geographic pest area		Natural controllers	Number and selected references
				Origin	Presence		
Caterpillar pest American bollworm	<i>Helicoverpa armigera</i>	Lepidoptera: Noctuidae	Wide range, including sunflower	Old world	Worldwide	<i>Chrysoperla carnea</i> , <i>Trichogramma chilonis</i> , beetles, spiders <i>Trichogramma</i> spp.	6, including Sanheep and Brar (2003), Ballal and Singh (2003)
Sunflower beetle	<i>Zygogramma exclamationis</i>	Coleoptera: Chrysomelidae	Restricted to <i>Helianthus</i> spp.	North America	North America	Coccinellidos, Carabidos, Tachinidos. <i>Myiopharus macellus</i>	3, including Brewer and Charlet (2004)
Caterpillar pest	<i>Spodoptera litura</i>	Lepidoptera: Noctuidae	Polyphagous	Old world	Worldwide	Several parasities	2, including Reddy et al. (2005)
Sunflower midge	<i>Contarinia schulzi</i>	Diptera: Cecidomyiidae	Restricted to <i>Helianthus</i> spp.	North America	North America	No record	Hodgson et al. (2004)
Red sunflower seed weevil	<i>Smicronyx fluvus</i>	Coleoptera: Curculionidae	<i>Helianthus</i> spp., <i>Veronica</i> sp., <i>Heliopsis</i> sp.	North America	North America	<i>Bracon</i> sp., <i>Nealiolus</i> sp., <i>Trimeromicrus</i> spp., <i>Torymus</i> sp., <i>Thereva</i> sp., <i>Rucifera</i> sp. <i>Triaspis aequoris</i>	Charlet (2002)
Banded sunflower moth	<i>Cochylis arthur</i> and <i>Cochylis hospes</i>	Lepidoptera: Cochylidae	Restricted to <i>Helianthus</i> spp.	North America	North America	<i>Orius</i> sp., <i>Glypta</i> sp., <i>Chelonus</i> sp. <i>Beauveria bassiana</i> y <i>Metarhizium anisopliae</i>	Foster et al. (2003)
Long-horned sunflower stem girdler	<i>Dectes texanus</i>	Coleoptera: Cerambycidae	<i>Ambrosia</i> sp., <i>Xanthium</i> sp. sunflower, soybean	North America	North America	Seven species of Hymenoptera	Michaud and Grant (2005)
Western corn rootworm	<i>Diabrotica virgifera virgifera</i>	Coleoptera: Crysomelidea	Corn, sunflower	America	America, Europe	<i>Beauveria bassiana</i>	Mulock and Chandler (2001), Horvath and Attila (2003) Yakutkin (2003)
Stem borer	<i>Mordellistena parvula</i>	Coleoptera: Mordellidae	Wide range	Eastern Europe	Central and Eastern Europe	No record	Yakutkin (2003)
Thrips	<i>Thrips palmi</i>	Thysanoptera: Thripidae	Wide range	Southeast Asia	India	Unspecific predators	Satish et al. (2004)
Bihar hairy caterpillar	<i>Spilosoma oblique</i>	Lepidoptera: Arctiidae	Wide range, very destructive in jute <i>Corchorus</i> spp.	Unknown	India	Wasp, including 4 <i>Apanteles</i> spp.	Arora et al. (2003)
Wireworms click beetles	<i>Agriotes</i> sp.	Coleoptera: Elateridae	Polyphagous	Depends on species	Worldwide	Predated by Carabidae and birds	Trasca et al. (2004)
Bug	<i>Nysius natalensis</i>	Hemiptera: Orsillidae	Wheat, grasslands, onion, sunflower, alfalfa	Hawaii	South Africa	No record	du Plessis et al. (2005)
Tenebroid beetle	<i>Opatrum sabulosum</i>	Coleoptera: Tenebrionidae	Wide range	Unknown	East Europe	Ants, beetles	Trotus (2003)
Percevejo	<i>Xyonysius major</i>	Heteroptera: Lygaeidae	Sunflower	Brazil	Brazil	No record	Aguiar et al. (2002)
Weevil	<i>Hypurus</i> sp.	Coleoptera: Curculionidae	Beans, sunflower, others	Mediterranean area	India	No record	Kumar (2001)

The general information of each pest was taken from Charlet et al. (1997).

the domestication of sunflower has reduced the biological control of its American relative, *Homeoesoma electellum*, in a clear example of a tritrophic relationship. Adult females easily lay eggs in big sunflower flowers, while parasitic Hymenopteran *Dolichogenidea homoeosomae* females find it difficult to do the same and prefer the smaller wild *Helianthus* flowers (Chen and Welter, 2003).

The most frequent methods used for insect control in sunflower involve the use of pesticides. A number of chemical products are recommended to control insects that reduce crop stand. Biotechnology could improve this control by helping to develop insect-resistant GM sunflower. However, sustainable management calls for a complete knowledge of the biology of the target pest and its relationship with other components of the agro-ecosystem.

New control options offered by genetic engineering include GM crops that express gene fragments from insecticide proteins of *Bacillus thuringiensis* (Bt endotoxins called Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, Cry1Fa, cry3Aa, and others), the *Vigna unguiculata* trypsin inhibitor (CpT1), lectins, and other metabolic inhibitors. The most widespread Bt proteins show strong activity against Lepidoptera, although some bacterial variants have also proven effective also against Diptera (*B. thuringiensis* var. *israeliensis*) and Coleoptera (*B. thuringiensis* var. *tenebrionis*). There is strong specificity in the action and expression Bt endotoxin. Not all the genes that codify Bt proteins are expressed in the different plant species. Similarly, nor all the Lepidoptera found in a crop are controlled by the same event.

On the other hand, the CpT1 agent is very active against Coleoptera and Orthoptera (Boulter et al., 1989) and is already available in GM crops. Modern biotechnological strategies incorporate the expression of a carrier to improve the toxin penetration and its influx into the insect's haemolymph (Fitches et al., 2004). The ideal GM technology should be environmentally friendly, with a wide spectrum of activity with respect to the target insects, but with few if any effects on beneficial insects (Hilder and Boulter, 1999).

The GM sunflowers released into the environment and authorized for research include two groups of events for insect control. The reported Lepidoptera-resistant varieties express the Bt insecticide protein, which is codified by the Cry1F gene. If expressed in the early stages of crop development, this could be a valuable tool for controlling polyphagous moth larvae of genera *Agrotis* and *Euoxa*, which are present in the main sunflower growing regions (Charlet et al., 1997). For *Suleima helianthana*, which bores sunflower roots and stems in North America, control through the use of GM varieties is difficult to justify because the damage caused is seldom significant (Charlet and Brewer, 2001).

Bt proteins could also offer excellent possibilities for controlling insect damage to aerial tissues. Lepidoptera that cause important crop damage include *Heliothis* spp., *Helicoverpa* spp., *Diabrotica* spp., *Spilosoma* spp., *Colias lesbia*, *Rachiplusia nu*, and *Vanessa cardui*. These species could be controlled through GM technology based on Cry1 variants of the Bt gene. As these species are highly polyphagous, refuges to

prevent the selection pressure for insect resistance would not be indispensable, except in cases where all the crops in a given region were GM varieties with the same expression of Bt proteins.

On the other hand, CpT1 could improve stand establishment in cases in which failure is due to Coleoptera of genera *Agriotes* sp., *Melolontha* sp., *Anoxia* sp., and Orthoptera of *Calolampra* spp. and *Teleogryllus* spp. Larvae of these species exhibit subterranean habits and eat plant roots at different stages of crop development, causing the death of seedlings in early attacks (Charlet et al., 1997; Lopez Bellido, 2002). To achieve the required impact at crop establishment, the expression of CpT1 toxin should take place early in crop development and involve concentrations that are lethal for the plague. Seedlings are very sensitive to the loss of certain of their parts, so it is therefore important to stop damage as early as possible at the beginning of the attack.

Two beetles cause economically important damage in North America (Charlet and Brewer, 2001). *Cylindrocopturus adspersus* mainly causes crop damage by lodging in weakened plants whose stems have been bored; this also facilitates the development of fungi. This pest can be controlled through the application of insecticides, though it would also be interesting to explore the genetic resistance of many wild sunflower species. A similar situation occurs with another Coleoptera, *Smicronyx fluvus*, whose larvae develop inside seeds. This is an oligophagous species, which is adapted to only a few hosts and can be controlled with insecticides, sometimes in combination with crop traps. Some parasitic Hymenoptera and Diptera act as controllers, and genetic resistance could therefore be achieved. Females consume head bracts and pollen before oviposition, so the expression of the CpT1 gene in these tissues would help to reduce adult populations. However, the probability of transgene escape points to the need for management strategies that limit the induction of insect resistance and the acquisition of transgenes by other wild host plants. This would provide durable resistance without environmental impact.

At present, control of insects that affect crop establishment is achieved through systemic insecticides that are preventively applied to the seed. Such products offer protection through rejection. One of the most popular of these products is imidacloprid, which has proven utility for the control of soil Elaterids (Pons and Albajes, 2002). However, it was withdrawn from the market in France because it causes honey toxicity for human and was associated with bee mortality, following the consumption of pollen from treated sunflower crops.

3.4. The *Sclerotinia* problem

Conventional plant breeding combined with simple management techniques offers a successful way to control most forms of disease affecting sunflower. However, stalk rot and head rot (white rot) which are caused by *Sclerotinia sclerotiorum*, one of the most important diseases affecting the crop worldwide, have yet to be effectively controlled in this way. Chemical control is only recommended to prevent disease from spreading through

seeds. Biological control has yet to be successfully applied, but appears to offer some promise (Elad, 2000). An algorithm using climate data can be used to determine the risk threshold for *Sclerotinia* in oilseed rape (Makowski et al., 2005) but there is so far nothing similar available for sunflower.

The causal agent responsible for white rot is a polyphagous fungus which attacks many plants, including soybean. The sclerotia, a fungus-resistant tissue, remain viable in the soil for up to 5 years. Under favourable environmental conditions attacks begin in the roots and stalk or in the head, depending on the stage of crop development. Although there is no the evidence of complete resistance among commercial hybrids (Pedraza et al., 2004) there have been continuous efforts to develop methods enabling early selection (Vuong et al., 2004) and lines with combining ability that help to obtain it (van Becelaere and Miller, 2004).

A biochemical disease study provided hints as to how to control this problem using biotechnological techniques. Research involving the heads of infected plants has shown that tolerance to white rot is related to the accumulation of phenolic compounds (Prats et al., 2003) and to the absence of the phytotoxic effect of oxalic acid (Baldini et al., 2002). The concentration of oxalic acid increases when tissues are damaged and this can be used as an indirect method for selecting on the basis of tolerance to disease (Vasic et al., 2002).

Biotechnology offers a number of strategies for the control of white rot (Schnabl et al., 2002), including defence activation, fungus inhibition, and detoxification (Lu, 2003). GM sunflower might present resistance to damage caused by *Sclerotinia* through over-expression of the oxalate oxidase (OXO) enzyme which degrades oxalic acid to carbon dioxide and hydrogen peroxide as a hypersensitivity mechanism. The first strategy outlined for sunflower by Lu et al. (1998) was also successful in other host plants. Donaldson et al. (2001) demonstrated that wheat gene expression of the OXO enzyme in soybean cells walls close to the site of pathogen attacks reduced disease progression.

The OXO effect in sunflower seems to be more than a hypersensitivity mechanism. Hu et al. (2003) demonstrated that fungus-related damage promotes defence gene activation that is independent of cell death in GM plants that express the wheat OXO gene. The transgenic event TF28 significantly improves white mold resistance in cultivated sunflower (see supporting online material in Burke and Rieseberg, 2003). The OXO expression may also reduce the herbivory action of certain insects, as demonstrated in maize under field conditions (Ramputh et al., 2002).

3.5. Product quality

Biotechnology offers other potential improvements in the quality of sunflower products and by-products. The fatty acid composition of some sunflower varieties has been modified through conventional plant breeding and mutagenesis (Lacombe and Bervillé, 2000). Although biotechnology could overcome some of the restrictions in this area and pave the way for further advances (Rousselin et al., 2002), its acceptance by

the consumer market must also be carefully considered. The high price of sunflower oil is due to it being perceived as a healthy, high quality product. Given that consumers in many countries are opposed to GM food, diffusion of GM varieties would probably affect its price and make sunflower products less popular than soybean alternatives.

After the oil extraction process, the residual sunflower meal has a low value as feed due to the limited level of methionin, an amino acid that is also scarce in other plant products. The Brazil nut (*Bertholletia excelsa*) is an exception to this general rule, providing high concentrations of this amino acid. Its genome has been biotechnologically manipulated in order to improve its amino acid content (Marcellino et al., 1996) and enable it to be transferred to other species. Unfortunately, Brazil nut albumen causes allergy in the natural product and also in GM soybean expressing its traits (Lack, 2002). Given that the *Codex alimentarius* (ftp://ftp.fao.org/esn/food/guide_plants) strongly recommends avoiding the transference of genes that cause allergies, interest in this kind of product for food purposes has declined. The situation for sunflower might however be different because the main destination of its meal is animal feed.

Interest in procuring alternative sources of latex has led to a search for increased biosynthesis in sunflower. The goal of this project, which has been exclusively sponsored by a governmental organization, is to commercially produce substitutes for USA imports. The guayule (*Parthenium argentatum*) is a desert shrub that produces a variant of rubber which does not cause allergy and which therefore has a high economic value. Progress in understanding the regulation of rubber biosynthesis in guayule has made it possible to obtain GM plants that offer profitable yields (Cornish and Scott, 2005; Veatch et al., 2005). The proposal for GM sunflower aims to achieve expression of the latex biosynthesis complex in sunflower (McMahan, 2006) because such an annual crop should facilitate extensive management.

4. The environmental impact of transgenes

The impact of gene flow from a GM crop depends on the expression of the transgene in the recipient population and how that modifies its fate (Darmency, 1994; Jorgensen et al., 1999). In each case, analysis is very complex due to the event in question and the environmental conditions at the location where the release will take place. At present there is a generally perceived need for in-depth, case-by-case research that takes into account the worst case scenario as a previous step to GMO release into the environment.

A consult to experts (FAO, 2005b) concluded that it was necessary to adapt the evaluation methodology for assessing the environmental impact of transgenic crops to the specific conditions of each agricultural system. In the EU the proposed guidelines for evaluating GM plants include a description of related species, the environment in which they occur, and the potential for interaction with other organisms within the agroecosystem (EFSA, 2004). The USA government control agencies are currently developing a cooperative study to

standardize the initial trials for GM ecological risk evaluation (Hellmich et al., 2005).

Gene flow in sunflower can reach 1000 m due to insect pollination (Arias and Rieseberg, 1994) and crop genes persist in wild populations for a long time (Linder et al., 1998). In the USA hybridization between GM sunflower and wild populations would be likely because more than half of the crops flower at the same time as wild *Helianthus annuus* subsp. *annuus* populations. At locations with similar flowering times, 10–33% of hybridization has been recorded, with the risk depending on the fitness consequences of the transgene (Burke et al., 2002). The crop can also hybridize with other species of its genus providing at least a few fertile progeny (Rogers et al., 1982). *Helianthus petiolaris* is an example of how crop gene flow can have an impact on wild relative populations (Rieseberg et al., 1999).

Transgene acquisition could have a reproductive cost, but this is not always negative. The Cry1Ac gene expression of Bt in wild *Helianthus* plants reduced damage due to Lepidoptera, thus increasing fecundity (Snow et al., 2003). This would increase seed production in wild populations expressing the Bt gene in environments in which target herbivores are the limiting factor. Furthermore, although they did not exhibit consistent resistance to disease, wild plants backcrossed to GM sunflowers expressing the OXO gene with conferred resistance to white rot did not have modified seed production in the presence of *Sclerotinia* (Burke and Rieseberg, 2003). This finding indicates that this transgene would not represent a reproductive cost for wild plants but would confer an adaptive advantage in environments in which disease was present.

No negative effects relating to insecticide protein expression have been reported on non-target Arthropoda (NTA); there are therefore no grounds on which to question the application of this biotechnology. Even so, a careful selection process should be conducted with species which could be affected by GM sunflower release expressing endotoxines. Scholte and Dicke (2005) proposed selecting and testing 4–6 NTA from the most relevant items in food-webs, on the basis of a number of pre-determined characters. The probable effects of endotoxines on soil fauna through root exudates should also be monitored (Saxena et al., 2004).

At the centre of origin, crop-wild hybridization constitutes a primary risk in the use of GM sunflower varieties in view of the modification of wild species of the genus that are useful as a natural germplasm reserve for breeding. A number of *Helianthus* species established in other continents merit other considerations. In Europe there are feral populations of *Helianthus tuberosus* and *Helianthus annuus* that could probably spread to the natural environment (Faure et al., 2002). The former is a hexaploid species which crosses with diploid cultivate sunflower, producing highly sterile F1 derivatives that exhibit numerous meiotic abnormalities. Crop progenies are volunteers which do not pose any risk “per se”, because they do not establish as durable populations. They are only found in fields in which sunflower has been seeded during the previous year and near road and rail transport routes. However, in areas where sunflower coexists with wild relatives,

volunteers can potentially act as transgene reservoirs and as a bridge for exchanges between cultivated and wild *Helianthus* (Reagon and Snow, 2006). Appropriate control may be necessary to prevent escapes. The recent discovery of established *Helianthus annuus* subsp. *annuus* populations in some sunflower crop regions in southern Spain (Bervillé et al., 2004) might change this situation in Europe.

The east coast of Africa seems to be a relatively new site for the recombination of genus *Helianthus* under field conditions, with intense gene flow. Established populations of *Helianthus argophyllus* and *Helianthus debilis* in Mozambique show a high frequency of hybrids (Vischi et al., 2004). In a similar way, in Argentina naturalized populations of *Helianthus annuus* subsp. *annuus* and *Helianthus petiolaris* hybridize with cultivated sunflower (Poverene et al., 2004a,b). Natural selection pressure outside their centre of origin possibly reduces interspecific reproductive barriers. The increased likelihood of hybridization could generate new biotypes. Populations that easily hybridize with the crop would be potential receptors of these transgenes which could give them some adaptive advantages. To prevent transgene escape, it is necessary to design appropriate strategies for each particular scenario. The best way to prevent gene flow between transgenic rubber producing sunflower and wild relatives would be to use male-sterile plants, which are currently under experimentation (McMahan, 2006).

Germplasm banks are invaluable tools for mitigating the consequences of crop gene flow on wild sunflower resources. In addition to the North Central Regional Plant Introduction Station at Ames, Iowa, USA several other countries including Serbia and Montenegro, Bulgaria, Ukraine, and India also preserve wild sunflower collections.

5. Conclusions

Most of the available transgenes and modulators that have been engineered could be expressed in different crops. However, in sunflower the traits that are being studied for environment release are limited and mainly consist of insect resistance, herbicide tolerance, and special compound synthesis. Leaving aside product marketing considerations, sunflower crops would greatly benefit from the introduction of GM varieties.

GM sunflower release at its centre of origin would have a major impact on wild populations of the genus among which hybridization is a distinct possibility. The marked oligophagy of some Arthropoda that affect the crop contributes to a high risk scenario with respect to insecticide proteins of GM sunflower potentially becoming expressed in wild sunflowers. The probable acquisition of herbicide-tolerant genes could be considered in a different way. A suitable management strategy, involving other herbicides than those associated with the GM event to control wild species, should reduce the risk of resistance developing in these populations. In this sense, tolerance to glufosinate-ammonium in GM sunflower would be promising if it were alternating with the glyphosate-tolerant RR soybean.

The scenario is different in certain areas in Europe, where there are no established wild populations and the risk of environmental impact deriving from insect resistance or herbicide tolerance would be minimal. Even so, as sunflower is a target crop for bees, GM events should ensure that there is no transgene expression in pollen or nectar in order to avoid contaminating honey.

In regions where naturalized *Helianthus* spp. populations coexist with the crop, the risk of transgene escape is very high. In these conditions, experiments should be designed to predict the potential consequences of transgene acquisition and their environmental impact. In non-native environments of the genus, the specificity of Arthropoda controllers and their natural enemies – which are not yet known – may not be as complex as at the centre of origin; this should facilitate the study of GM expressed insecticide proteins. As far as weed control is concerned, the general situation and management considerations should be similar to those mentioned above.

Transgenic events related with sunflower products quality do not suppose any competitive advantage with respect to GM plants and would probably have a low environmental impact. Under present market conditions the only viable event would seem to be hypoallergenic guayule latex. Acquisition of these transgenes by wild *Helianthus* species would be unlikely to destabilise the ecosystem. Even so, it should be remembered that sunflower is a species with a high exposure to gene flow and which continuously generates variability. In view of this, it is necessary to ensure strict environmental monitoring in order to prevent any potentially undesirable outcomes.

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